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Nitric oxide synthase inhibition ameliorates nicotine-induced sperm function decline in male rats

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ABSTRACT

Objectives: To evaluate the effects of inhibiting nitric oxide synthase as a means of intervention in nicotine-induced infertility in male rats.

Methods: Forty-eight male and thirty female Wistar rats (180–200 g) were randomly assigned to six groups and treated orally for 30 days with saline (control), nicotine (0.5 mg/kg, 1.0 mg/kg) with or without NG Nitro-L-Arginine Methyl Ester (L-NAME, 50 mg/kg). Treated male rats were cohabited with untreated females in ratio 1:2 for fertility studies. Sperm analysis was done by microscopy.

Results: There was a significant decrease in the epididymal sperm motility and count after nicotine treatment. However, the percentage of abnormality significantly increased in nicotine treatment groups. Fertility studies revealed that nicotine reduced libido in male rats and decreased litter weight and number delivered by the untreated female during the experiments. Co-treatment with L-NAME effectively reversed the nicotine-mediated alterations in the sperm functional parameters, fertility indexes and hormone when compared to nicotine only.

Conclusion: Taken together, the present data indicate the abilities of L-NAME to ameliorate nicotine-induced spermatotoxic effects in male rats *via* a mechanism dependent on the circulating testosterone level.

1. Introduction

In the last decade, nitric oxide (NO) which is a highly reactive free radical gas and reactive oxygen species (ROS) has assumed an important functional role in a variety of physiological systems and different pathways, therefore it is indisputable that such a polyvalent molecule should also play a decisive role in the reproductive system.

NO is synthesized from an essential amino acid L-arginine by a family of isoenzymes known as the nitric oxide synthases (NOS) [1].

Basal generation of NO plays an important role in the physiology of several organs. Studies have shown that in the vascular system, NO inhibit platelet aggregation, induce vasodilation, prevent neutrophil/platelet adhesion to endothelial cells, maintain endothelial cell barrier function and inhibit smooth muscle cells proliferation and migration [2].

NO was first recognized in the reproductive system by Ignarro *et al*. [3], who demonstrated that NO was generated in response to non-adrenergic/non-cholinergic neurotransmission-mediated penile erection. Following this finding, several other studies have implicated NO in its involvement in penile erection at several neuronal levels [4]. It has long been documented that regulation of penile erection by androgens and the pituitary and its relationship with NOS activity is of special physiological interest and deficiency of male sex hormone such as testosterone, has long been associated with impotence and dysfunction of penile erection [5,6]. Studies have shown that castration in rodents results in a significant decrease in NOS activity in the penis and significantly reduced electrical stimulation-induced penile erection [6,7].

Furthermore, in the female reproductive system, it was demonstrated that expression of NOS was increased in the cervix, and decreased in the uterus, during labour and preterm labour [8]. NO also seems to be involved in pre-eclamptic conditions and pregnancy-related hypertension [8].

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NO has also been shown to regulate sperm motility. Lewis *et al*. [9] documented that low concentrations of NO have been shown to enhance sperm motility while Rosselli *et al*. [10] concluded that high concentrations of NO decrease sperm motility.

The use of nicotine seems to remain a broad public health concern since several million of humans use nicotine worldwide through smoking for a prolonged period of time and infertility among couples of child bearing age is also on the rise. In spite of the growing knowledge of effects of NO on reproduction and the association between nicotine and male reproductive dysfunction, little is known about the effect of inhibiting NOS and its effect on nicotine-induced infertility, Therefore, this present study was designed to investigate whether or not inhibition of systemic biosynthesis of nitric oxide will ameliorate nicotine-induced infertility in rat models.

2. Materials and methods

2.1. Animals

The Experiments were performed on forty-eight male and thirty female Wistar rats, 2–2.5 month old and whose average weight ranged between 180 g and 200 g obtained from the Animal House, College of Medicine, University of Ibadan, Oyo State, Nigeria. Animals were divided into six equal groups with *ad libitum* access to rat chow and drinking water. Animals were also maintained in a well-ventilated room with a 12/12-h light/dark condition at room temperature. The experiment was conducted in accordance with the Guidelines of the U.S. National Institute of Health (NIH) on the care and use of laboratory animals. The male animals in the six groups were treated orally for 30 days and they included the control group that received 0.2 ml/kg normal saline, 0.5 mg/kg nicotine-treated group, 1.0 mg/kg nicotine-treated group, 50 mg/kg L-NAME, 0.5 mg/kg nicotine alongside with 50 mg/kg L-NAME and 1.0 mg/kg nicotine alongside with 50 mg/kg L-NAME.

2.2. Drug preparation

2.2.1. Nicotine preparation

Nicotine hydrogen tartrate (95% Nicotine) (BDH Chemicals Ltd., Poole, England) was used in the study. The nicotine dosage freshly prepared in normal saline for each group of animals was delivered at 0.5 mg/kg and 1.0 mg/kg per body weight. The working solutions were stored in foil-wrapped glass bottle at 4 °C for no longer than ten days.

2.2.2. Nitric oxide (NO) synthesis inhibition

*N*G-nitro-L-arginine methylester (L-NAME) (Sigma Chemicals St Louis, MO, USA), a nitric oxide synthase (NOS) inhibitor was administered in the drinking water at a dose calculated to provide 50 mg/kg/day to rats. This was administered in light-proof bottles for a period of 4 weeks. It was used to determine the role of NO synthesis in nicotine induced infertility.

2.3. Sperm characteristics analysis

The left testis was removed along with its epididymis. The caudal epididymis was separated from the testis and lacerated to collect the semen with a microscope slide for semen characteristics evaluation as previously described [12].

Progressive motility was tested immediately. Semen was squeezed on a pre-warmed slide, two drops of warm 2.9% sodium citrate was added to it. This was then covered with a cover slip, examined and scored under the microscope using ×40 objective with reduced light [13]. A viability study (percentage of live spermatozoa) was done using eosin/nigrosin stain. Semen was squeezed onto a microscope slide and two drops of the stain were added. The motile (live) sperm cells were unstained while the non-motile (dead) sperms absorbed the stain. The stained and the unstained sperm cells were counted using ×40 microscope objectives and an average value for each was recorded from which percentage viability was calculated. Sperm morphology was evaluated by staining the sperm smears on microscope slides with two drops of Walls and Ewas stain after they were air dried. The slides were examined under the microscope under oil immersion with ×100 objective. The abnormal sperm cells were counted and the percentage calculated according to the method described by Wyrobek and Bruce [14]. The epididymis was immersed in 5 mL normal saline in a measuring cylinder and the volume displaced was taken as the volume of the epididymis. Sperm count was done under a microscope with the aid of the improved Neubauer hemocytometer. Counting was done in five Thoma chambers [15].

2.4. Libido test

To observe the libido-oriented mounting behaviour, non-oestrous untreated female rats were paired on the 30th day at 6.00 pm. The male rats assuming the copulatory position over the female rats, but failing to achieve intromission was considered as a mount [16]. Male rats from each group were chosen and suitably marked. The rats were placed in a clear aquarium and were allowed to acclimatize for 15 min. Afterwards a non-oestrous female rat was introduced into the arena. The number of mounts was recorded for 15 min. This process was also done for the recovery groups.

2.5. Fertility studies

A total of thirty untreated fertile, proestrous female rats were used for the fertility test. Five untreated female rats were cohabited with a male rat from one of the six male groups on the 31st day of treatment. All animals were cohabited for 5 days according to earlier studies [16]. The presence of a vaginal plug was accepted as the index for a positive mating and it was taken as day one of pregnancy [17]. A fertility test was calculated using the following formula: [18]

$$\%\ \text{Fertility success} = \frac{\text{Pregnancy female} \times 100\%}{\text{Mated female}}$$

The number of litters delivered and their body weights were determined.

2.6. Statistical analysis

Data are expressed as means ± S.E.M. for each group. One-way analysis of variance (ANOVA) was used to analyse for significance of difference between means followed by post hoc